characteristics are produced when the expression of a particular gene is reduced or inhibited.

METHODS

Tissue Samples

[0128] The cDNAs of the current invention can be derived from any sets of plant tissue. The cDNAs of SEQ ID NOS: 1-334, for example, were originally derived from embryonic tissues of pine tree embryos staged 1-9.9 as classified in Pullman and Webb TAPPI R&D Division 1994 Biological Sciences Symposium, pages 31-34, which is hereby incorporated by reference. LPS and LPZ clones are derived from somatic and zygotic embryos, respectively. As noted, embryos may be of either somatic or zygotic derivation, and the embryos may be grown in either semi-solid or liquid tissue culture systems. Applicable methods for growing embryos in semi-solid or liquid tissue culture systems are disclosed in U.S. Patents: 5,036,007; 5,236,841; 5,294,549; 5,413,930; 5,491,090; 5,506,136; 5,563,061; 5,677,185; 5,731,203; 5,731,204; and U.S. Patent Application 60/212,651 filed June 19, 2000, which are hereby incorporated by reference.

RNA Isolation

[0129] In one embodiment, RNA isolated from staged cell populations provides the starting material for reverse transcription, differential display, and cloning of amplified cDNA. Methods and kits for isolating total RNA from cellular populations, or for generating poly(A)+ RNA, are commonly known in the art. For example, several procedures for isolating RNA are disclosed in Chapter 4 of *Current Protocols in Molecular Biology* edited by F.A. Ausubel et al., John Wiley and Sons, Inc. (1987)

(incorporated herein by reference). As an example, the TRI Reagent7 available from Molecular Research Center, Inc. (Cincinnati, OH) is a suitable reagent (used according to the manufacturer's instructions) for isolation of RNA from plant tissues.

Differential Display

[0130] Differential display provides a method to identify individual messenger RNAs that are differentially expressed among two or more cell populations. In the practice of the present invention, these cell populations may be provided by pine tree or other plant embryos of different developmental stages. The differential display procedure is taught in Liang et al., *Science*, 257:967-71 (1992) and in U.S. Patent No. 5,262,311, which are hereby incorporated by reference. Briefly, mRNA sequences are PCR-amplified using two types of oligonucleotide primers known as "anchor" and "arbitrary" primers. Anchor primers are designed to recognize the polyadenylate tail of messenger RNAs. Arbitrary primers are short and arbitrary in sequence and anneal to complementary sequences in various mRNAs. Products amplified with these primers will vary in size and can be differentiated on an agarose or sequencing gel based on their size. If different cell populations are amplified with the same anchor and arbitrary primers, one can compare the amplification products to identify differentially expressed RNA sequences.

Cloning

[0131] PCR-amplified bands representing differentially expressed RNA samples are excised from the gel, transferred to tubes and reamplified using the same primer pairs and PCR conditions as used in the differential display procedure. Methods for the cloning of PCR products are commonly known in the art and there are several

commercially available reagents and kits for cloning PCR products. For instance, the pCR-Scipt™ Cloning kit from Stratagene, La Jolla, California) is suitable for this purpose. Using this kit, *E. coli* transformants containing plasmids with PCR fragment inserts can rapidly be identified using blue/white color selection followed by plasmid purification and restriction digests. The pCR-Script vector contains T3 and T7 polymerase recognition sites allowing for in vitro transcription of the inserted fragment.

Sequence Determination

[0132] Methods for sequencing DNA, including cloned PCR products, are commonly known in the art. The selection of cloning vectors having M13, T7 or T3 primer annealing sites flanking the PCR-amplified insert can be used in sequencing reactions directly. Most sequencing procedures in use today are modifications of Sanger's dideoxy chain termination sequencing reaction as disclosed in and Sanger et al., *Proceedings of the National Academy of Sciences*, 74:5463-5467 (1977), which is hereby incorporated by reference.

Homology Searching and Identification of Protein Coding Sequences

[0133] As understood by one of ordinary skill in the art, the sequence of a cloned cDNA insert obtained, may be compared against public databases such as Genbank to discern any identity or homology to known sequences. Programs, such BLAST, for performing such a search are available on the National Center for Biotechnology Information's web page located at http://www.ncbi.nlm.nih.gov. The results from Genbank search may reveal the potential function of a polypeptide or RNA molecule encoded by the cDNA. In addition to searching gene sequence database, the use of commercially available analysis software is well known in the art. For example,